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### *Pandoraea apista* in mild lung disease

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*Pandoraea apista* is newly described as a highly transmissible pathogen in CF causing respiratory deterioration. It may be misidentified as *B. cepacia* complex. Cohort isolation is recommended. **Methods:** Sputum samples are grown on conventional media and *B. cepacia* selective medium (Mast Diagnostics, United Kingdom). Following identification techniques are used: conventional phenotypic tests, whole-cell fatty (CFA) analysis with Microbial identification System (MIS, Microbial ID, Newark, Del), DNA sequencing of the 16S rRNA gene and SDS-Page of whole cell proteins. **Results:** A 20 yrs old CF male (E60X/ΔF508) with mild disease has *Pseudomonas aeruginosa* isolation since 1999, treated with colimycin aerosols. In 2004 *S. aureus* and *P. aeruginosa* are cultured next to a rod-shaped gram-negative bacterium on a B. cepacia-selective medium. Biochemical and CFA analysis matches with *Pandoraea* species. *Pandoraea apista* is identified by comparison of its whole-cell protein profile with those of references strains of a range of Gram negative non-fermenting bacilli. Phenotyping and DNA sequencing of the 16S rRNA gene could not differentiate between *Pandoraea apista* and *Pandoraea pulmonicola.* Patient is isolated. **Discussion:** Identification of new CF pathogens is challenging because of their differences in pathogenic potential and transmissibility. Identification may be problematic in routine laboratories, and increase workload of microbiology units. However *B. cepacia* showed the importance of correct identification in a CF unit, to avoid spread. Mostly clinical suspicion is impossible. Cohort isolation becomes complicated, and impossible without a qualified laboratory.

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### Chlamydia pneumoniae infection and cystic fibrosis

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**Objectives:** The aim of this study was to evaluate whether Chlamydia pneumoniae might be involved in cases of adult patients with cystic fibrosis.
**Materials and methods**: The studies were performed on 24 cystic fibrosis adults, aged 18 – 38 years and in control group, 15 patients (aged 19-42) after acute upper airways infection. Fibreoptic bronchoscopy was performed in all patients and segmental bronchi samples of mucous membrane were taken. DNA was isolated from mucous membrane samples, using QIAamp DNA Mini Kit (Qiagen). Presence of the isolated DNA was checked electrophoretically. Chlamydia pneumoniae (147-bp) sequence of DNA were detected using nested PCR amplification assay. Detection was carried out in 2% agarose gels using ethidium bromide.
**Results**: Chlamydia pneumoniae DNA was detected in 7 cystic fibrosis patients (29,2%) and in 2 patients from control group (13,3%).
**Conclusion**: The results indicate that Chlamydia pneumoniae may be involved in the clinical course of cystic fibrosis patients.

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### Bacteraemia with Gram negative non-fermenting bacilli in CF

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The last decade several Gram-negative non-fermenting bacilli (GN-NFB) other than *Pseudomonas aeruginosa* are newly described in CF, some as highly transmissible pathogens causing rapid respiratory deterioration. *B. cepacia* complex is best documented, however bloodstream infections are uncommon in CF. The latter may be increasing as reported in hematology and intensive care units. **Methods:** A total of 129 pediatric and adult patients are treated in our center, 26.2% are colonized by *P. aeruginosa*, *B. cepacia* is present in only 4 patients. Sputum samples are grown on conventional media and *B. cepacia* selective medium (Mast Diagnostics, UK). Blood cultures are performed in patients with persisting fever or chills despite correct antibiotic treatment. **Results:** All blood cultures remained negative, except in 2 patients: a 29 yrs old male and a 27 yrs old female both on a lung transplantation waiting list. Both were infected with *B. cepacia*, and had an intravascular catheter. *B. cepacia* was cultured repeatedly from their blood samples. Moreover *B. cepacia* was isolated again in one patient after removal of the infected catheter and replacement of a second long-term catheter. *P. aeruginosa* was never recovered in any of the CF blood samples, even in patients with longstanding intravascular catheter and end-stage respiratory failure. **Discussion:** GN-NFB septicaemia may be increasing due to better identification techniques, older ages, aggressive treatment and the use of long-term catheters. Mortality may be high. It is unclear why only GN-NFB blood infections were seen in our series.

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### *B.cepacia* participation in respiratory infections in Cystic Fibrosis in Greece

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**Objectives** *B.cepacia* is considered a threatening pathogen for CF patients. In Greece, isolation frequency of *B.cepacia* among microorganisms causing serious respiratory infections in CF patients is considered extremely rare. We investigated the presence of *B.cepacia* in sputa of CF patients
**Methods** The selective medium *B. cepacia* agar (Oxoid) was used, in addition to conventional media, for quantitatively culturing sputum samples of CF patients, admitted to our Hospital, for the period 2003-2004. Identification methods included API 20E, API NE, and Vitek 2 (Biomerieux). All Gram (-), oxidase (+) isolates were also screened initially by a multiplex PCR, in order to characterize the two bacterial species *P. aeruginosa* and B. *cepacia*. The multiplex PCR included primers specific for the 520bp fragment of the *algD* GDP mannose dehydrogenase gene of *P. aeruginosa*, and the 463bp fragment of the ribosomal 16S gene, identifying the five main gemovars of *B. cepacia*. Included in the multiplex PCR was a universal bacterial primer pair targeting the 16S rRNA to act as internal control (233bp). Samples giving results for the 520 and/or 463bp fragments were further tested for the identification of the bacterial strain with specific PCR. The recA gene PCR is a useful assay, which discriminates between the *B. cepacia* complex and *B. cepacia* like microorganisms.
**Results** None of Gram negative isolates was identified as *B. cepacia*, either by using the selective *B.cepacia* medium or performing PCR.
**Conclusions** There is strong evidence that *B. cepacia* participation in CF respiratory infections is rather unusual in Greece.